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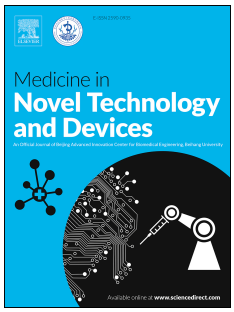

Review of Ex-vivo Characterisation of Corneal Biomechanics

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Review of Ex-vivo Characterisation of Corneal Biomechanics

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Abstract
The evaluation of the corneal biomechanical behaviour has important clinical applications. To name a few, the accuracy of the intraocular pressure measurement, the study of corneal ectatic diseases and the assessment and optimisation of corneal surgical procedures are all highly influenced by corneal biomechanics. Over the last 45 years different ex-vivo methods were developed to study corneal biomechanical behaviour. Different tissue maintenance, support, loading systems, as well as different monitoring strategies of corneal deformations were employed. In this review, the most important and commonly used methods are outlined, including strip extensiometry, inflation, compression, indentation and tissue separation testing. Their particularities, applications, pros and cons and main applications are discussed.

1. Introduction

The biomechanical behaviour of the cornea is important to a number of clinical applications including the planning of refractive surgery procedures, the selection of corneal rings, following up keratoconus progression, optimisation of the cross-linking treatment and the measurement of intraocular pressure (IOP), to name but a few. Recognition of the importance of corneal biomechanics and the role it can play in these and other applications can be traced back to publications in the 1970s [1]. Efforts to characterise corneal biomechanics started with ex-vivo tests that grew in complexity with the appreciation that the tests needed to approximate the organ's physiologic conditions for the results to be meaningful. Over the last forty years, significant progress has been made in the way tissue is maintained, supported and loaded and in how the tissue's deformation was monitored, recorded and used to estimate the biomechanical properties. Much has also been achieved in estimating the cornea's stiffening with age [2, 3] and diseases such as diabetes [4], and softening with keratoconus [5, 6].

The cornea is a structurally complex, viscoelastic membrane with regionally different and anisotropic biomechanical properties. Up to now, strip extensometry has remained the most commonly-used method to evaluate corneal biomechanics due to its simple process and low cost – despite its several drawbacks. However, due to these drawbacks, other methods have been introduced including: inflation, compression, indentation, lamellae separation, shear and other testing methods [7]. This paper presents a review of the methods used to derive the cornea's biomechanical properties, their pros and cons, and the applications each method would be suitable for.

2. Strip extensometry testing

Strip extensometry is probably the simplest and most commonly used ex-vivo testing method to determine the stress-strain behaviour of corneal tissue [8]. The method involves separation of a rectangular strip of tissue, with constant width, and subjecting it to uniaxial tension while monitoring its elongation, Figure 1. The experimental results, namely the applied load, P, and the resulting elongation, δ, are converted into stress and strain, respectively, by dividing the load by the specimen's cross-sectional area, and dividing the elongation by the initial, unloaded, specimen length. This simple analysis yields the stress-strain behaviour depicting the material behaviour, which is intended to be independent of the specimen's length, thickness or width. The tangent to the stress-strain relationship at any stress or strain then produces the tangent modulus, which is considered the best measure of stiffness at that particular stress or strain.

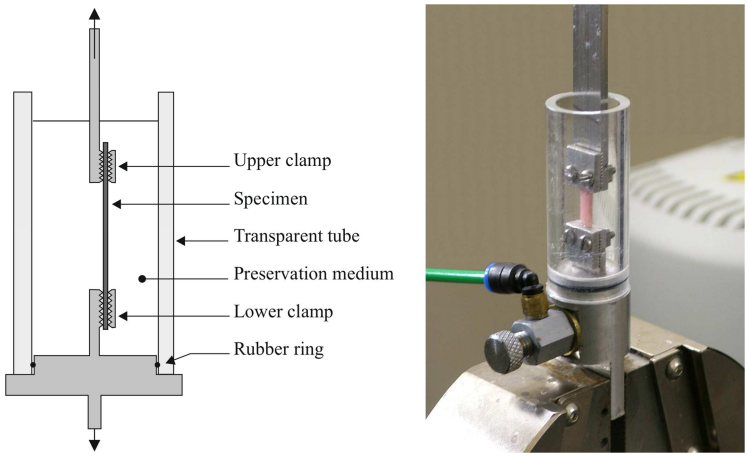


Figure 1 Setup and main components of a strip test rig – preservation medium is not shown in picture

The test method involves several inherent deficiencies, which should be considered carefully [9]. Two of the deficiencies originate from the fact that the strip specimen is originally part of the 3D curved surface of the cornea. As a result, the length of the specimen along its longitudinal centreline is longer than its length along the edges. This variation in specimen length inevitably leads to a non-uniform strain distribution across the width of the specimen with the centreline undergoing lower strains than the specimen edges, Figure 2. For a cornea with an anterior central radius of curvature of 7.8 mm, anterior shape factor of 0.8, central corneal thickness of 0.55 mm, peripheral corneal thickness of 0.70 mm and rotational symmetry, the difference in length between the centreline and the edge of a 2 mm wide specimen that extends the full cornea width (limbus to limbus) is 1.7%, which will affect the distribution of strain and stress across the specimen width.

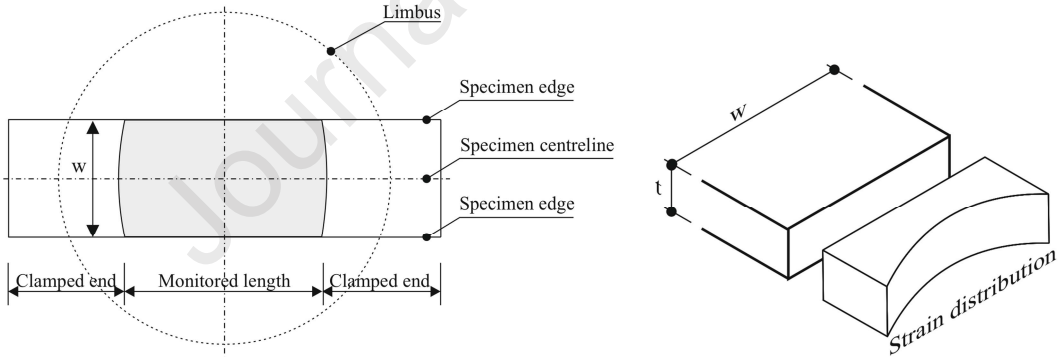


Figure 2 Strain distribution under axial load is not uniform due to variation in specimen length from maximum along the centreline to minimum along the edges

The second deficiency is caused by the flattening of the initial curved specimen as it produces tensile strains, and stresses, on the posterior side and compressive strains on the anterior side, Figure 3 [9]. These initial strains could be considerable even if corneal thickness is considered small in relation to the other dimensions. For the example cornea mentioned above, the strain caused by the tissue flattening would be around 1.9% at both the anterior and posterior surfaces. As a result, the initial compressive and tensile strains generated respectively during flattening on the anterior and posterior sides will lead to a non-uniform stress distribution under the applied tension load.

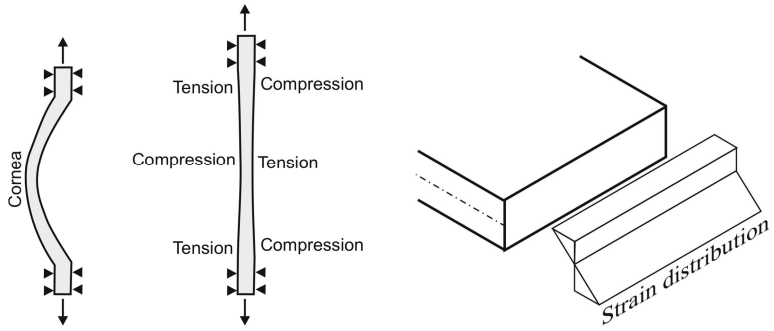


Figure 3 Strain distribution caused by straightening the initially curved corneal strip specimen

A further complication could arise due to the use of mechanical clamps at specimen ends and the low shear cohesion between the cornea's stromal lamellae. It has been suggested that the clamping effect could be stronger at the outer lamellae, allowing the internal lamellae to slip relative to the clamps and leading to further effect on the uniformity of stress distribution under the applied tensions loads [10].

The literature also shows that the analysis method of the test results usually considers the central corneal thickness or the average specimen thickness in deriving the material stress–strain behaviour [11, 12]. While this practice leads to a very simple analysis method, it ignores the fact that corneal thickness is not constant, but varies from a minimum at or near the apex and increases gradually towards the limbus, Figure 4. The thickness profile is even more complex in ectatic corneas where local thinning is expected at the area of pathology. An attempt to resolve this issue, and hence improve the stress calculations was presented in an earlier study [9]:

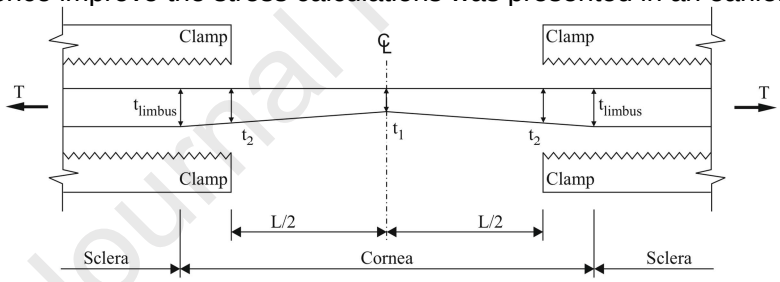


Figure 4 Variation of specimen's cross-sectional area from a minimum at the midpoint to a maximum at the endpoints

$$E = \frac{L\,T}{\delta\,A_1}\,\frac{t_1}{t_2 - t_1}\,[\ln(t_2\,l) - \ln(t_1\,l)] \qquad (1)$$

where T is the applied tension force, L specimen length, l half specimen length, δ specimen elongation, A1 cross-sectional area at specimen mid-point, t1 and t2 specimen thickness at midpoint and endpoint, respectively. Note that the first term (L T/δ A_1) in Equation (1) represents the value of E when assuming constant cross-sectional area, A_1, while the second term $\left(t_1/(t_2 - t_1)\right)[\ln(t_2\,l) - \ln(t_1\,l)]$ is a correction factor to take account of an assumed linear change in thickness from t_1 at midpoint to t_2 at endpoints.

On a microstructure level, the preparation of a strip specimen inevitably entails severing of collagen fibrils that cross the specimen edges. The resulting disruption of the tissue's microstructure, which controls its biomechanical behaviour, is therefore likely to affect the reliability of the measured mechanical properties of the tissue.

These deficiencies have contributed to the perception that strip extensometry testing was a less reliable procedure to determine the cornea's material properties than inflation testing [13-15].

However, their relative simplicity and low cost encouraged the use of strip tests in several studies from the 1980s until present [16]. While it is appreciated that the technique was not reliable in estimating the material's stress-strain behaviour, it was thought to remain adequate for comparative studies in which the focus is on the effect of certain parameters on material behaviour. Example applications include estimating the deterioration in corneal stiffness with keratoconus [5, 17], the increase in stiffness with the cross-linking treatment [18], the effect of biomechanical anisotropy [19, 20] and the effect of viscoelasticity [21, 22].

Attempts to improve unidirectional strip testing are described below and included biaxial tension tests in which square tissue specimens are dissected and loaded in tension in two orthogonal directions. This technique is more compatible with the anisotropic nature of corneal tissue but still suffers from most of the drawbacks of unidirectional testing including those caused by flattening the initially curved specimens and severing collagen fibrils along specimen ends. The technique is also significantly more expensive than unidirectional testing – a feature that led to its rare use in previous research studies [23, 24].

3. Inflation testing

Awareness of the deficiencies of strip testing has led to increased use of inflation testing despite the large difference in cost. Inflation testing keeps the tissue intact and loads it with an internal pressure that simulates the intraocular pressure, Figure 5. The pressure is applied through fluid injection using a water column or a syringe pump that can be computer-controlled in a closed loop with the aid of a pressure transducer feedback signal measurement. In tests involving the cornea, specimens are connected to clamps along their rings of scleral tissue. Similarly, in tests of the sclera, the specimens are clamped along the limbus and the anterior part of the tissue. Both cornea and sclera tests involve dissecting the tissue and using non-physiologic supporting conditions. For this reason, there has been a move towards whole globe testing to avoid the negative effects of the supports on specimen behaviour [25]. In that case, the internal pressure is applied through a hypodermic needle inserted into the anterior chamber or, more commonly, the posterior ocular cavity.

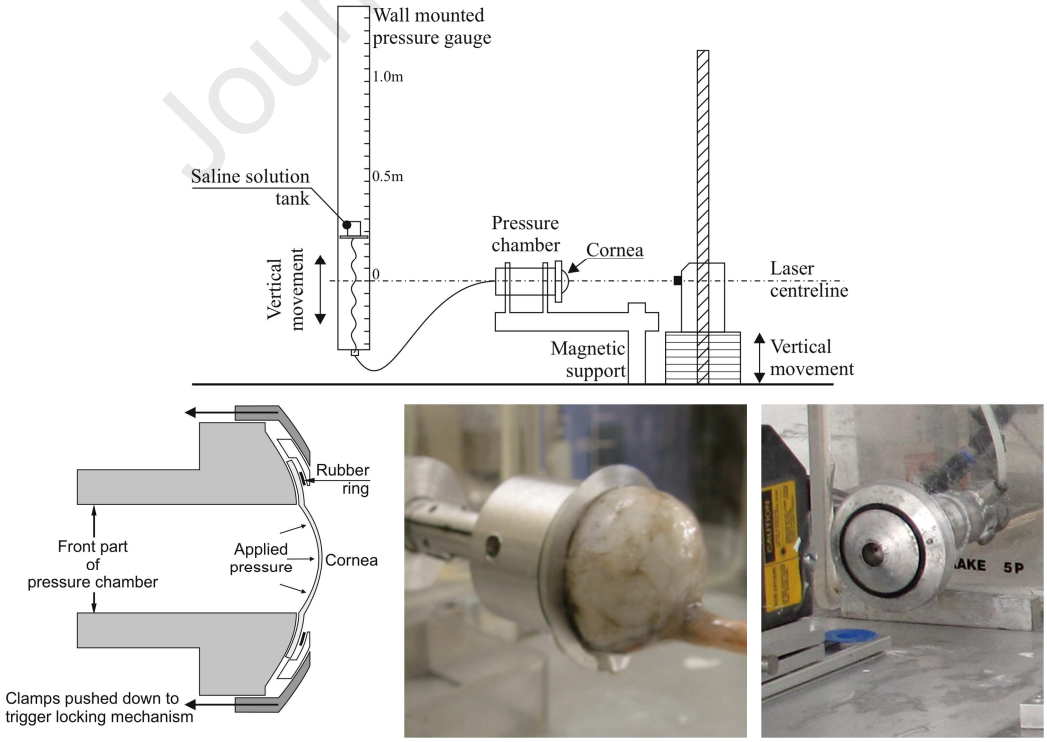


Figure 5 Layout of a simple inflation test setup (top), design of a cornea clamp (bottom left), and images of a human sclera connected to a pressure chamber (bottom middle) and being subjected to an internal pressure simulating intraocular pressure (bottom right)

The deformation of the specimens that results from changing the internal pressure is measured by a system of high-resolution, digital cameras that are spatially distributed around the specimen [25, 26]. The camera images, taken under different internal pressures, are analysed using digital image correlation (DIC) systems to quantify the displacement distribution across the surface of the specimens. Other imaging methods that have been used with success include the Scheimpflug camera technology (the Pentacam video keratographer) and the optical coherence tomography (OCT) [27-29]. Laser beams are also used to measure the displacement at specific locations, including corneal apex, the posterior pole or points on the limbus, Figure 5 [3]. Due to the higher accuracy of the laser beams, relative to DIC and other imaging systems, laser beam measurements have also been used to check and validate the output of other systems.

The initial specimen dimensions, the applied pressure and the measured deformations could be analysed using shell analysis to determine the stress-strain behaviour of the tissue [2, 26], Figure 6. The analysis starts with the initial conditions where the cornea's median surface is assumed to be spherical with an initial radius of $R_o{}^2 = R_i{}^2 + (R_o - H_o)^2$ or $R_o = \left(R_i{}^2 + H_o{}^2\right)/(2\,H_o)$, where R_i is the limbal radius and H_o is the initial corneal height. With the first pressure application, p_1, the initial corneal height (H_o) increases by the apical rise recorded experimentally: $H_1 = H_o + r_1$ and the corresponding radius becomes: $R_1 = \left(R_i{}^2 + H_1{}^2\right)/(2\,H_1)$.

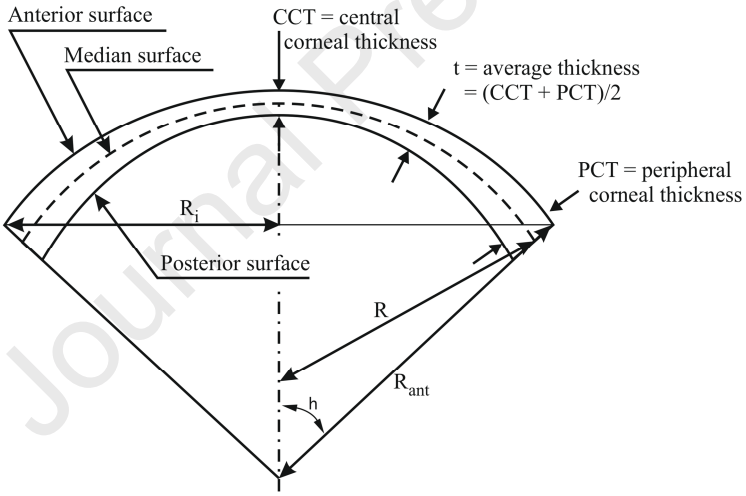


Figure 6 Schematic cross-sectional view of the cornea under inflation with the main dimensions

Assuming the tissue is incompressible, leading to no change in volume with loading, the new average tissue thickness becomes $t_1 = (R_o\,H_o\,t_o)/(R_1\,H_1)$, and the angle h becomes $h_1 = \sin^{-1}(R_i/R_1)$. With these values calculated at the first pressure application, the corresponding tangent modulus is obtained as:

$$E_1 = \frac{p_1\,R_1{}^2}{2\,r_1\,t_1}\,(1-\nu)\left\{1 - e^{-\beta h_1}\cos(\beta h_1)\right\} \quad (2)$$

The corresponding strain is then calculated as:

$$\varepsilon_1 = \frac{p_1\,R_1}{2\,E_1\,t_1}\,(1-\nu)\left\{1 - \nu\,e^{-\beta h_1}\cos(\beta h_1)\right\} \quad (3)$$

and the corresponding stress: $\sigma_1 = E_1\,\varepsilon_1$. This process is then repeated for every pressure increase to derive the stress-strain relationship of the tissue and the tangent modulus at each stress or strain.

Now, more commonly, the stress-strain behaviour of the tissue is obtained using the finite element based inverse analysis process. This process avoids the inherent simplifications of the shell analysis including, most notably, the assumption that the cornea has a spherical shape. In inverse analysis, the initial camera images taken under zero internal pressure (or low pressure

just enough to maintain an inflated specimen shape) are used to construct a numerical model of the specimen that adopts its outside geometry and thickness distribution. The model is then subjected to a trial and error process (called inverse analysis) while changing the tissue's material behaviour until a close match is achieved between the model's deformation predictions and the experimental measurements. This method has higher accuracy than the shell analysis described above as it does not require any assumptions in tissue thickness distribution, supporting conditions or corneal geometry. The inverse analysis method further enables dividing the test specimen into segments, each with its unique material constitutive model. However, as a trial and error process, the reliability of inverse analysis in producing unique estimations of the material behaviour reduces with more constitutive models considered.

A further advantage of inflation testing over strip extensometry is their respective response to preconditioning. Preconditioning is a process, by which specimens are subjected to cycles of loading and unloading up to the maximum load/pressure intended in the actual tests, and using the same load/pressure application rate, in order to reach a stage of stable behaviour. In strip extensometry, and with the flattening of the tissue and severance of collagen fibrils along the specimen edges, preconditioning results in successive permanent deformations due to the gradual realignment of the fibrils. These deformations affect the resulting estimated behaviour of the specimen and adds another element of uncertainty to a long list of strip extensometry deficiencies. Inflation testing, on the other hand, does not suffer the same drawback as the loading simulates closely the intraocular pressure and therefore preconditioning does not entail significant behaviour changes.

Inflation testing was used in numerous studies to determine the material biomechanical properties of healthy, diseased and treated eyes and how the properties change with age and certain treatments. Examples include studies on the age-related stiffening of corneal tissue [2, 30-32] and scleral tissue [33, 34]. Inflation testing was also used to evaluate if Bunsen-Roscoe law was applicable in corneal cross-linking (CXL), and showed evidence that the effect of CXL in stiffening the tissue decreased with reducing the irradiance duration [35]. Besides, it was used to investigate the biomechanical effects of Travoprost and Tafluprost on the rabbit cornea, and demonstrated significant reductions in tangent modulus with both forms of PGF2α [9]. The stiffness-reduction effect of fluorometholone on the rabbit cornea was also reported previously using inflation testing [36], and diabetes was shown to induce significant increases in biomechanical stiffness as evidenced by increases in tangent modulus [4].

4. Compression testing

Compression tests can be performed with the tissue specimen being either confined or unconfined (Figure 7). Confined compression testing measures the capacity of a specimen to withstand a surface compressive force while not allowing in-plane expansion – perpendicular to the force. These tests are usually performed on biphasic materials, such as those with poroelastic properties. The unconfined compression test, on the other hand, is usually known as the uniaxial compression test. In this case, the applied force causes a compressive stress across the thickness, and this stress causes both reduction in thickness and in-plane expansion to preserve tissue volume. This test is usually employed to characterize the biomechanical properties of biological tissues under compression.

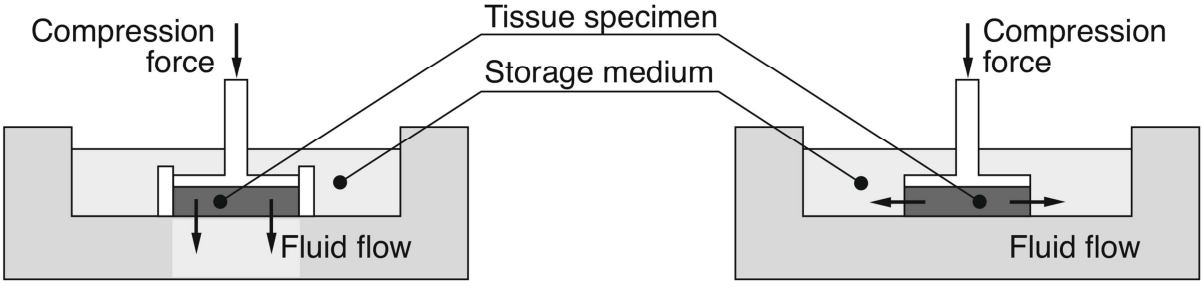


Figure 7 Confined (left) and unconfined (right) compression test setups

Unconfined compression testing has been conducted in the past using displacement-controlled test rigs, although load-control could also be used [37-40]. Cornea disc specimens were kept in a bathing solution of preservation medium and subjected to a compression force across the thickness. The pressure-displacement behaviour recorded was fitted to a linear transversely isotropic biphasic model, in which the cornea was considered as a mixture of an incompressible solid phase (collagen lamellae, proteoglycans, and keratocytes) and an incompressible fluid phase (interstitial fluids, and ions) [40]. However, the analysis could still be done simply by dividing the applied force by the specimen surface area to determine the applied stress, and dividing the tissue thinning by the specimen's initial thickness to calculate the corresponding strain.

Indentation testing

This form of mechanical testing is one of the simplest and easiest to implement. It involves pushing an indenter with a spherical [41] or a flat tip [42] against the cornea while monitoring both the indentation force and the tissue's deformation, Figure 8. Indenters with a wide range of diameter, between 1.7mm and 5.4mm, were used. The test method was used on whole eye globes, usually ex-vivo, although it can be used on separated corneas or smaller tissues, and can also be implemented in-vivo while following a process similar to that followed with the Goldmann Applanation Tonometer.

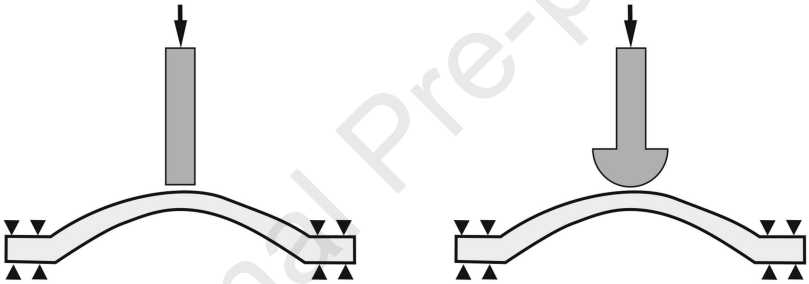


Figure 8 Indentation tests using indenters with a flat tip or a spherical tip

Analysis of the experimental force and deformation results was carried out while following different methods. In the case with a spherical indenter [42], the tangent modulus of the tissue, E_t, was estimated as:

$$E_t = \frac{3\,F\,(1-\nu^2)}{4\,x^{3/2}\,\sqrt{R}} \tag{4}$$

where F is the indentation force, X the resulting specimen displacement under the indenter, v Poisson's ratio of the tissue and R the radius of the indenter. This equation lacks reference to the tissue's thickness, which undoubtedly has a significant effect on behaviour under indentation. It also refers to an analytical method developed for plane surface under indentation, and hence ignores the effect of the cornea's curvature [43].

For the case with flat tip indenters [44], the tissue's tangent modulus was estimated using:

$$E_{IOP} = \frac{a\,(R-t/2)\,\sqrt{1-\nu^2}}{t^2}\,\frac{dF}{d\delta}\Big|_{IOP} \tag{5}$$

Where F is the indentation force, delta the indentation depth, v Poisson's ratio, R anterior corneal radius of curvature, t the central corneal thickness and a is a geometry constant which can be determined from the parameter μ:

$$a = 0.0349\,\mu^3 - 0.1034\,\mu^2 - 0.0291\,\mu + 0.434 \quad (6)$$

where

$$\mu = r_o \left[\frac{12\,(1-\nu^2)}{(R-t/2)^2\,t^2}\right]^{1/4} \quad (7)$$

and r_o is the radius of the indenter.

While these equations present a simple method to calculate the tangent modulus, they embody simplifications related to the thickness variation and corneal curvature – both assumed to be constant. An alternative method that can avoid these simplifications is based on inverse analysis, similar to what has been presented for inflation testing.

5. Lamellae separation tests

This form of testing has been little used to assess the interlamellar adhesive strength in corneal tissue [45]. Strips of tissue are removed from cornea specimens and their ends slit to separate the anterior and posterior stromal layers. The two ends are then connected to a uniaxial testing machine to apply forces leading to the gradual tear in the tissue and progress of the separation between the layers, Figure 9.

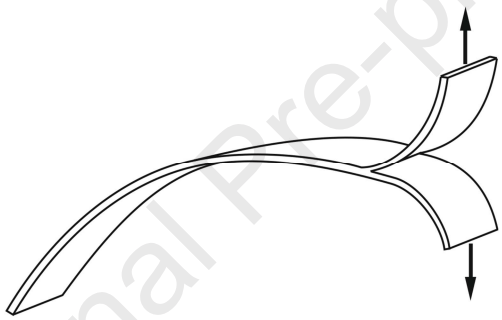


Figure 9 Strip specimen used in a lamellae separation test

The technique was used in a recent study to determine the cohesive tensile strength within the corneal stroma [46]. The study found that the anterior 40% of the central stroma had the highest cohesive strength, whereas the posterior 60% of the stroma was weaker by more than 50%. Another study used the technique to measure the cohesive tensile strength of human LASIK corneal wounds and found that corneal stroma typically heals after LASIK in a limited and incomplete fashion, resulting in a weak, central and paracentral stromal scar that averaged 2.4% as strong as normal corneal stroma [47].

Overall, it is difficult to control the load rate or the elongation rate applied in separation tests as they are sensitive to the direction of the fibrils of the test tissue. As the tearing progresses, it also becomes impossible to keep a right angle between the peeling force and the specimen's separation line.

Shear testing

This form of testing is used to measure the transverse shear stiffness of the corneal stroma and its variation through the tissue thickness [48, 49]. Shear testing takes two different forms subjecting the tissue to in-plane torsion in rheology testing [48], or direct in-plane shear forces [50], Figure 10. The results of both set ups confirmed the hypothesis that the shear stiffness was greater in the anterior third of the stroma compared with the remainder of the tissue thickness due to the increased interweaving of anterior lamellae. The results further reported shear moduli that were two to three orders of magnitude lower than what would be estimated for isotropic materials, for which the shear modulus, G was equivalent to E/(1 - ν). This finding was compatible with the observation of stromal lamellae easily sliding against each other [51, 52].

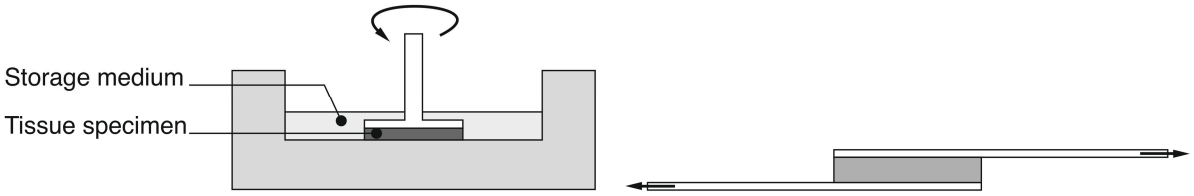


Figure 10 Main components of a rheology shear test setup (left), and a direct shear test setup (right)

6. Discussion

Biomechanical testing of ex-vivo corneal tissue has contributed much to our understanding of corneal biomechanics. The quantification of the age-related stiffening of the tissue, the stiffening caused by diabetes and the softening caused by keratoconus are outcomes of ex-vivo testing. The testing has also helped characterise the regional variation of biomechanical behaviour, the tissue's viscoelasticity and anisotropy, the stromal shear stiffness and the epithelium's contribution to corneal biomechanics. These results have improved in accuracy as the test methodologies matched closer the tissue's physiologic conditions including how it was supported and loaded.

However, despite the improvements in ex-vivo testing, difficulties remained in translating tissue behaviour obtained in ex-vivo experiments into behaviour of in-vivo corneas [53] due to possible tissue degradation and the wide variation in behaviour between individual eyes. This has led to a growing interest in in-vivo measurement of corneal biomechanics, in particular over the last two decades. This point is discussed in detail in our accompanying paper reviewing the development of in-vivo corneal test methods. However, as interest in corneal biomechanics and the role it can play in the customisation of treatments and the follow up of disease progression, it is expected that ex-vivo testing will maintain its importance and continue to offer insight into tissue behaviour especially in disease and under treatment.

The difficulty in obtaining human tissue specimens at the required numbers made it necessary in some cases to rely on animal models, primarily porcine and rabbit tissue. Evidence has shown that these models provided similar behaviour to human tissue and were therefore useful in preliminary studies. However, over the years evidence based on human tissue tests has accumulated and provided clear pictures of the human cornea's hyperelasticity, viscoelasticity and anisotropy, reducing the need for animal models.

Recognition of the dependence of corneal biomechanics on the tissue's microstructure has enabled the development of detailed understanding of tissue behaviour that has been impossible in the past. The parallel progress of microstructure research and biomechanics characterisation is expected to accelerate in the future leading to unprecedented ability to customise treatments and take clinical practice into the domain of multi-scale numerical modelling.

The ex-vivo evaluation of corneal biomechanical properties – especially in cases where in-vivo assessment is not possible – could provide useful information to optimize several treatments of corneal conditions. Applications could include customization of collagen cross-linking (CXL) to focus on areas of pathology, selection of implants used to improve visual acuity in eyes with irregular corneas, and improving risk profiling to reduce likelihood of ectasia post refractive surgery.

Different specimen maintenance, support and loading systems, as well as different monitoring strategies of corneal deformation, have been developed with successively improved matching of the cornea's physiologic conditions and hence improved prediction of corneal biomechanics. Strip extensometry testing has advantages in terms of simplicity, low cost and ability to provide a quick, quantitative measure of stiffness. However, its drawbacks made it suitable only for

comparative studies such as assessing the effect of specific treatments on behavior, and less reliable for quantifying the stress-strain performance of the tissue. The drawbacks also made it necessary to develop other methods, most notably inflation testing, to keep the cornea intact and simulate its complex physiologic conditions. Other methods have also been developed to estimate specific biomechanical properties of corneal tissue including the compression, shear and lamellae cohesion tests. Combinations of these tests are usually required to derive the corneal properties needed for specific applications.

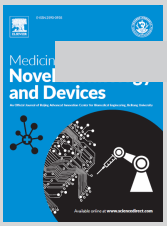
While successes are being achieved in the in-vivo determination of corneal biomechanics, ex-vivo testing remains essential for understanding tissue behavior and the effect of disease progression and treatment on performance. Despite the advances made over the last few decades to make ex-vivo testing more representative of the tissue's physiologic conditions, progress is still needed to streamline the currently expensive test protocols and improve the accuracy of the estimated material parameters.

References

[1] SL. W, AS. K, WA. S, C. L. Nonlinear material properties of intact cornea and sclera. Exp Eye Res 1972;14(1):29-39.
[2] Elsheikh A, Wang D, Brown M, Rama P, Campanelli M, Pye D. Assessment of corneal biomechanical properties and their variation with age. Curr Eye Res 2007;32(1):11-9.
[3] Elsheikh A, Geraghty B, Rama P, Campanelli M, Meek KM. Characterization of age-related variation in corneal biomechanical properties. J R Soc Interface 2010;7(51):1475-85.
[4] Bao F, Deng M, Zheng X, Li L, Zhao Y, Cao S, et al. Effects of diabetes mellitus on biomechanical properties of the rabbit cornea. Exp Eye Res 2017;161:82-8.
[5] Andreassen TT, Simonsen AH, Oxlund H. Biomechanical properties of keratoconus and normal corneas. Exp Eye Res 1980;31(4):435-41.
[6] Scarcelli G, Besner S, Pineda R, Yun SH. Biomechanical characterization of keratoconus corneas ex vivo with Brillouin microscopy. Invest Ophthalmol Vis Sci 2014.
[7] Curatolo A, Birkenfeld JS, Martinez-Enriquez E, Germann JA, Muralidharan G, Palaci J, et al. Multi-meridian corneal imaging of air-puff induced deformation for improved detection of biomechanical abnormalities. Biomedical optics express 2020;11(11):6337-55.
[8] Ashofteh Yazdi A, Melchor J, Torres J, Faris I, Callejas A, Gonzalez-Andrades M, et al. Characterization of non-linear mechanical behavior of the cornea. Scientific reports 2020;10(1):11549.
[9] Elsheikh A, Anderson K. Comparative study of corneal strip extensometry and inflation tests. J R Soc Interface 2005;2(3):177-85.
[10] Elsheikh A, Brown M, Alhasso D, Rama P, Campanelli M, Garway-Heath D. Experimental assessment of corneal anisotropy. J Refract Surg 2008;24(2):178-87.
[11] He X, Liu J. Correlation of corneal acoustic and elastic properties in a canine eye model. Invest Ophthalmol Vis Sci 2011;52(2):731-6.
[12] Hoeltzel DA, Altman P, Buzard K, Choe K. Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas. J Biomech Eng 1992;114(2):202-15.
[13] Vellara HR, Patel DV. Biomechanical properties of the keratoconic cornea: a review. Clin Exp Optom 2015;98(1):31-8.
[14] Hibbard RR, Lyon CS, Shepherd MD, McBain EH, McEwen WK. Immediate rigidity of an eye. I. Whole, segments and strips. Exp Eye Res 1970;9(1):137-43.
[15] Buzard KA. Introduction to biomechanics of the cornea. Refract Corneal Surg 1992;8(2):127-38.
[16] Torres-Netto EA, Hafezi F, Spiru B, Gilardoni F, Hafezi N, Gomes JAP, et al. Contribution of Bowman's layer to corneal biomechanics. J Cataract Refract Surg 2020.
[17] Nash IS, Greene PR, Foster CS. Comparison of mechanical properties of keratoconus and normal corneas. Exp Eye Res 1982;35(5):413-24.
[18] Abdshahzadeh H, Abrishamchi R, Torres-Netto EA, Kling S, Hafezi NL, Hillen M, et al. Impact of hypothermia on the biomechanical effect of epithelium-off corneal cross-linking. Eye and vision 2021;8(1):4.
[19] Elsheikh A, Alhasso D. Mechanical anisotropy of porcine cornea and correlation with stromal microstructure. Exp Eye Res 2009;88(6):1084-91.

[20] Xue C, Xiang Y, Shen M, Wu D, Wang Y. Preliminary Investigation of the Mechanical Anisotropy of the Normal Human Corneal Stroma. J Ophthalmol 2018;2018:5392041.
[21] Elsheikh A, Kassem W, Jones SW. Strain-rate sensitivity of porcine and ovine corneas. Acta of bioengineering and biomechanics / Wroclaw University of Technology 2011;13(2):25-36.
[22] Liu T, Shen M, Li H, Zhang Y, Mu B, Zhao X, et al. Changes and quantitative characterization of hyper-viscoelastic biomechanical properties for young corneal stroma after standard corneal cross-linking treatment with different ultraviolet-A energies. Acta biomaterialia 2020;113:438-51.
[23] Kanellopoulos AJ. Comparison of corneal biomechanics after myopic small-incision lenticule extraction compared to LASIK: an ex vivo study. Clin Ophthalmol 2018;12:237-45.
[24] Hu Y, Huang Y, Chen Y, Ye C, Wei W, Feng Y, et al. Study on patterned photodynamic cross-linking for keratoconus. Exp Eye Res 2021;204:108450.
[25] Whitford C, Joda A, Jones S, Bao F, Rama P, Elsheikh A. Ex vivo testing of intact eye globes under inflation conditions to determine regional variation of mechanical stiffness. Eye and vision 2016;3:21.
[26] Boschetti F, Triacca V, Spinelli L, Pandolfi A. Mechanical characterization of porcine corneas. J Biomech Eng 2012;134(3):031003.
[27] Kling S, Remon L, Perez-Escudero A, Merayo-Lloves J, Marcos S. Corneal biomechanical changes after collagen cross-linking from porcine eye inflation experiments. Invest Ophthalmol Vis Sci 2010;51(8):3961-8.
[28] Wang L, Tian L, Huang Y, Huang Y, Zheng Y. Assessment of Corneal Biomechanical Properties with Inflation Test Using Optical Coherence Tomography. Ann Biomed Eng 2018;46(2):247-56.
[29] Lombardo G, Serrao S, Rosati M, Lombardo M. Analysis of the viscoelastic properties of the human cornea using Scheimpflug imaging in inflation experiment of eye globes. PLoS One 2014;9(11):e112169.
[30] Elsheikh A, Wang D, Rama P, Campanelli M, Garway-Heath D. Experimental assessment of human corneal hysteresis. Curr Eye Res 2008;33(3):205-13.
[31] Elsheikh A, Alhasso D, Rama P. Biomechanical properties of human and porcine corneas. Exp Eye Res 2008;86(5):783-90.
[32] Elsheikh A, Alhasso D, Rama P. Assessment of the epithelium's contribution to corneal biomechanics. Exp Eye Res 2008;86(2):445-51.
[33] Elsheikh A, Geraghty B, Alhasso D, Knappett J, Campanelli M, Rama P. Regional variation in the biomechanical properties of the human sclera. Exp Eye Res 2010;90(5):624-33.
[34] Geraghty B, Jones SW, Rama P, Akhtar R, Elsheikh A. Age-related variations in the biomechanical properties of human sclera. J Mech Behav Biomed Mater 2012;16:181-91.
[35] Bao F, Zheng Y, Liu C, Zheng X, Zhao Y, Wang Y, et al. Changes in Corneal Biomechanical Properties With Different Corneal Cross-linking Irradiances. J Refract Surg 2018;34(1):51-8.
[36] Yu JG, Bao FJ, Joda A, Fu XA, Zhou S, Wang J, et al. Influence of glucocorticosteroids on the biomechanical properties of in-vivo rabbit cornea. J Mech Behav Biomed Mater 2014;29:350-9.
[37] Hatami-Marbini H, Etebu E. Rate dependent biomechanical properties of corneal stroma in unconfined compression. Biorheology 2013;50(3-4):133-47.
[38] Hatami-Marbini H, Etebu E. An experimental and theoretical analysis of unconfined compression of corneal stroma. J Biomech 2013;46(10):1752-8.
[39] Hatami-Marbini H, Etebu E. Hydration dependent biomechanical properties of the corneal stroma. Exp Eye Res 2013;116:47-54.
[40] Hatami-Marbini H, Etebu E. A new method to determine rate-dependent material parameters of corneal extracellular matrix. Ann Biomed Eng 2013;41(11):2399-408.
[41] Nohava J, Swain M, Lang SJ, Maier P, Heinzelmann S, Reinhard T, et al. Instrumented indentation for determination of mechanical properties of human cornea after ultraviolet-A crosslinking. J Biomed Mater Res A 2018;106(5):1413-20.
[42] Lu SH, Chong IT, Leung SYY, Lam DCC. Characterization of Corneal Biomechanical Properties and Determination of Natural Intraocular Pressure Using CID-GAT. Translational vision science & technology 2019;8(5):10.

[43] Harding J, Sneddon I. The elastic stresses produced by the indentation of the plane surface of a semi-infinite elastic solid by a rigid punch. *Mathematical Proceedings of the Cambridge Philosophical Society.* 41. Cambridge University Press; 1945:16-26.
[44] Ko MW, Leung LK, Lam DC. Comparative study of corneal tangent elastic modulus measurement using corneal indentation device. Med Eng Phys 2014;36(9):1115-21.
[45] Tao C, Sun Y, Zhou C, Han Z, Ren Q. Effects of collagen cross-linking on the interlamellar cohesive strength of porcine cornea. Cornea 2013;32(2):169-73.
[46] Whitford C, Movchan NV, Studer H, Elsheikh A. A viscoelastic anisotropic hyperelastic constitutive model of the human cornea. Biomechanics and modeling in mechanobiology 2018;17(1):19-29.
[47] Schmack I, Dawson DG, McCarey BE, Waring GO, 3rd, Grossniklaus HE, Edelhauser HF. Cohesive tensile strength of human LASIK wounds with histologic, ultrastructural, and clinical correlations. J Refract Surg 2005;21(5):433-45.
[48] Hatami-Marbini H. Viscoelastic shear properties of the corneal stroma. J Biomech 2014;47(3):723-8.
[49] Petsche SJ, Chernyak D, Martiz J, Levenston ME, Pinsky PM. Depth-dependent transverse shear properties of the human corneal stroma. Invest Ophthalmol Vis Sci 2012;53(2):873-80.
[50] Elsheikh A, Ross S, Alhasso D, Rama P. Numerical study of the effect of corneal layered structure on ocular biomechanics. Curr Eye Res 2009;34(1):26-35.
[51] Dupps WJ, Jr. Biomechanical modeling of corneal ectasia. J Refract Surg 2005;21(2):186-90.
[52] Maurice DM. Some puzzles in the microscopic structure of the stroma. J Refract Surg 1999;15(6):692-4.
[53] Jester JV, Petroll WM, Garana RM, Lemp MA, Cavanagh HD. Comparison of in vivo and ex vivo cellular structure in rabbit eyes detected by tandem scanning microscopy. J Microsc 1992;165(Pt 1):169-81.



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All authors were responsible to review the most up-to-date literature on ex vivo measurement of corneal biomechanics and to write specifics parts of the manuscript.

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